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viewpoint

When a common problem meets an ingenious mind

The invention of the modern micropipette

Martin Klingenberg

When the marvels of modern biological research are presented in the media, one image almost always appears: a person in a laboratory using a micropipette. This small but ubiquitous device has evolved into one of the icons of modern biotechnology, molecular biology, gene therapy, stem-cell technology and cloning. The modern micropipette has achieved such high visibility for obvious reasons: it is without exaggeration, the most widely used instrument in biology and medicine. It enables the convenient and precise handling of very small liquid volumes, making it of paramount importance to most laboratory work, and has contributed significantly to the rapid progress of molecular biology. Despite its obvious importance, the micropipette has always been taken for granted and little is known about its origins.

I therefore want to tell the story of the invention of the piston-driven, plastic-tip micropipette. This singular feat was accomplished in the late 1950s by Heinrich Schnitger, a German scientist and inventor (Fig 1). Schnitger drowned in 1964 and his mentor Theodor Bücher passed away in 1997, so it is no longer possible to obtain a personal account of his invention. I was a close witness to Schnitger's work on the micropipette and other inventions, which took place at the Institute of Physiological Chemistry at the University of Marburg, Germany. In addition to my personal recollections, I rely on Birgit Pfeiffer's excellent research on the development of the 'Marburg pipette' (Pfeiffer, 2004).

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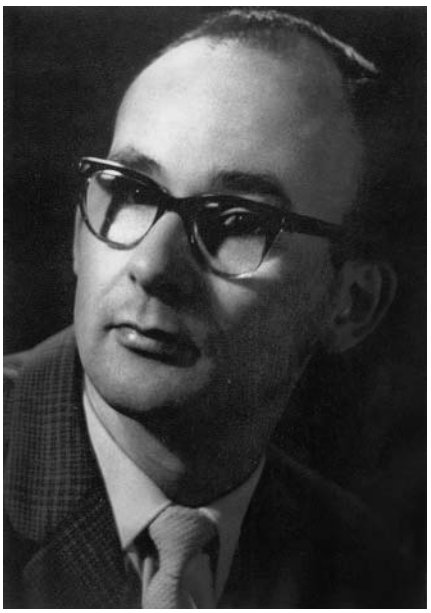

Fig 1 | Heinrich Schnitger at about 38 years of age. Courtesy of the Institute of Physiological Chemistry, Ludwig-Maximilians University, Munich, Germany.

Few people will remember how biologists handled microlitre volumes before the modern micropipette became available. On my arrival as a postdoc at the Johnson Foundation of the University of Pennsylvania (Philadelphia, PA, USA) in 1954, my first task was to construct a set of 'personal' micropipettes. These were based on the so-called Carlsberg pipette, which was made by heating a glass tube over a Bunsen burner and tugging at one end to create a capillary. Further heating a few millimetres from the tip of the capillary created a restriction that allowed air flow, but limited the flow of liquid to define the volume. The pipettes had to be calibrated gravimetrically with mercury or using a dye. Pipetting itself involved sucking the fluid by mouth up to the restriction—not surprisingly, precise pipetting required experience, and depended on the user and pipette construction. Those working in a well-funded laboratory could buy commercially available pipettes—Misco (Cleveland, OH, USA) sold a set of calibrated constriction pipettes of various volumes and small Pyrex vessels which fit into a microcentrifuge.

Not surprisingly, there were many drawbacks to the Carlsberg pipette. The use of filters to protect against ingesting toxic or infectious fluids was not a fail-safe precaution. Small particles often plugged the restriction and rendered the pipette unusable. Some enzymologists cleaned their micropipettes using poisonous chromic sulphuric acid to remove contamination. They broke easily, particularly the delicate tips, which were often chipped away. In addition to constriction pipettes, other devices were commonly used, such as single-use glass capillaries that filled spontaneously when dipped into a liquid. In the late 1950s, the first commercial micropipettes using metal pistons became available. However, because the liquid

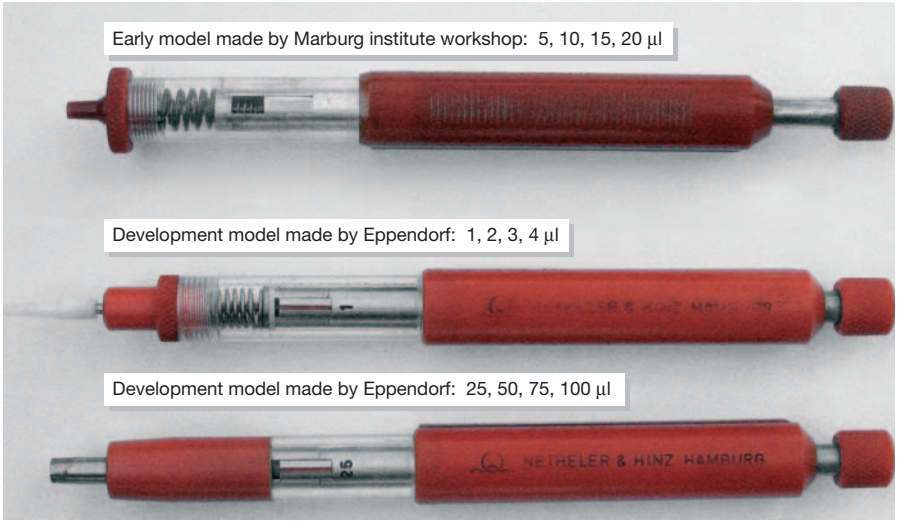

Fig 2 | Micropipettes from the first production series in 1957–1960. Photo by the author.

came into contact with the metal pistons, there was always the risk of corrosion and contamination. The pistons could also become jammed in the glass capillary, making cleaning cumbersome.

In 1956, I joined the group of Theodor Bücher, Director of the Institute of Physiological Chemistry at the University of Marburg (Germany) and pipetting by mouth was still the only method used. We made our own pipettes or used commercial ones from Misco. This was at a time when Bücher's group developed optical enzyme assays, which drastically increased the need for precise micropipetting as well as the volume of pipetting work. This project led to the development of various research and clinical assays to measure metabolite or enzyme concentrations in biological extracts, which quickly expanded as an increasing number of purified enzymes became available.

Heinrich Schnitger joined Bücher's group in 1957 as a postdoc and experienced the same pitfalls in handling microlitre volumes. His annoyance with the cumbersome Carlsberg pipette eventually led him to invent the modern micropipette and thus radically change the way in which biologists handle small volumes of liquids. His invention was not a sudden enlightenment; the vexing problem of micropipetting met an ingenious mind who challenged problems from an unconventional angle.

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Heinrich Schnitger was born in 1925 in Lemgo, Westphalia, Germany, the son of an inventor who designed, among other things, a once widely used bicycle lock. As a young boy, Schnitger had already tinkered with and made many changes and improvements to various gadgets. After suffering from tuberculosis as a soldier in the Second World War, which probably saved his life, he decided to study medicine. He assured me that this was not to practice medicine itself, but to control his health while protecting himself from incompetent doctors. During Schnitger's term at the University of Marburg's Medical School, he noticed how time-consuming it was to determine the time it takes for blood to coagulate, which was then done by hand. Schnitger developed an apparatus with two specially arranged wires as electrodes, which moved in and out of the serum until the clotted blood formed a permanent conduction between the wires; this then triggered a stop clock. Where the development teams of several major companies had failed, he alone succeeded in constructing a working device. The patented apparatus is described in Schnitger's dissertation (Schnitger, 1956). A small company produced his device until Becton, Dickinson & Co. (Franklin Lakes, NJ, USA) bought the license to build and market a modular version from 1960 onwards.

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When Schnitger joined Bücher's group in Marburg, he was assigned to work with the new anion exchange chromatography, used to measure phosphate-containing metabolites. A gradient of up to 80% formic acid, following gravity flow, separated nucleotides and other anionic metabolites, which were collected in up to hundreds of fractions, often less than a millilitre in volume, for further analysis. Within a few weeks and to everyone's surprise, Schnitger developed a piston-driven pumping system, which replaced the gravity-driven flow of acid by more exact pump-controlled pressure. The formic acid was stored in a flexible polyethylene (PE) bag placed in a metal chamber filled with glycol and, by using PE tubes for the pump itself and to link it to the chromatography column, the corrosive fluid came into contact only with inert plastic materials.

It was obvious from the outset that while doing his routine work of aliquoting chromatography fractions for further analysis, Schnitger viewed micropipetting by mouth with great contempt. He eventually disappeared from the laboratory for a couple of days and came back with a self-designed tool to pipette microlitre volumes. His device already had all the essential features of what would later become the modern micropipette. Initially, Schnitger 'rebuilt' a tuberculin syringe by adding a spring to the piston that met an upward stop to define the pipetting volume. The syringe needle was replaced by a PE tip, pulled from PE tubing. An air buffer separated the fluid from the syringe piston and confined it to the plastic tip. The device was originally intended for pipetting chromatography fractions that contained corrosive formic acid so that they did not touch the metal piston, but the clever features of Schnitger's device dramatically sped up and eased many other experiments, as it enabled more accurate pipetting of all aqueous solutions. Bücher soon realized the enormous potential of this invention and encouraged Schnitger to develop the pipette further while relieving him of his research work. Schnitger added various mechanical measures required for the exact and repetitive pipetting of small

volumes. A major breakthrough was the introduction of a second coaxial spring, which allowed the piston to be pushed beyond the delivery point to blow out any residual fluid from the plastic tip.

While Schnitger made the various parts of the micropipette himself, he also profited from the institute's excellent mechanical workshop, which was established by Bücher in the tradition of Otto Warburg. On the basis of Schnitger's prototypes, the workshop technicians produced copies for use in the laboratory (Fig 2). Shortcomings were detected and fed back to Schnitger for alterations and improvement. Six months after he had built his first prototype, and conscious of its importance, Schnitger applied for a patent in Germany. His application, dated 3 May 1957, entitled "Vorrichtung zum schnellen und exakten Pipettieren kleiner Flüssigkeitsmengen" (Device for the fast and exact pipetting of small liquid volumes), was finally granted on 24 April 1961 (Fig 3; German Patent Office, 1957, 1961). It describes all the essential features of the modern pipette, such as the spring-loaded piston, the second coaxial spring to blow out residual liquid and the replaceable plastic tip as the sole container of the liquids. Other aspects, such as precautions to keep an air-tight seal around the piston and using an enlarged piston to keep the air cushion small and thus decreasing errors from ambient temperature effects, were also explained. Schnitger's patent application also outlined the mechanics for pipetting variable volumes either by discrete steps or by continuous adjustments.

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Although the piston-driven pipette would eventually be used for larger volumes, Schnitger's real interest was in the exact and convenient pipetting of very small volumes in the microlitre range. He soon realized that the material and shape of the plastic tip were important for exact handling of liquids. Schnitger was fascinated by the properties of Teflon®, which he thought to be an excellent material for pipette tips because it completely repels water if the surface is smooth and clean. To obtain smooth surfaces, he made diamond-honed tools with polished cutting edges to carve micro-tips from solid Teflon. The idea was to deliver microlitre drops of liquid in such a way that the tip was left clean for using with other solutions. Since Teflon cannot be melted for injection molding, Schnitger experimented with baking Teflon powder into the shape of a micropipette tip. In tireless work he determined the optimum conditions for sintering the powder while developing special equipment to control pressure and temperature precisely. Even the manufacturer of Teflon, Dupont Inc. (Wilmington, DE, USA), sent its experts to Marburg to study this new sintering procedure. When properly handled, the tips were unsurpassed for accuracy. But they were too expensive for single use and required careful maintenance. Teflon tips were eventually used only during the early stages of the invention in Marburg and for the initial experimental production sets.

In anticipation of a broad market for the Marburg pipette, as it became commonly known—not only for research but also for medical applications—the medical supply company Eppendorf (Hamburg, Germany), bought the exclusive license for manufacturing and marketing the micropipette. Wilhelm Bergmann was responsible for developing the pipette for general use and large-scale production. For better handling, the micropipette morphed into new shapes, the original adjustment mechanism for four different volumes was omitted, and—most importantly—Teflon was replaced by the newly available polypropylene (PEP) to create the tip. The inexpensive, translucent and rigid PEP tip was ideal for single use. The shape of the pipette end was also modified to guarantee a tight fit with the tips. As an important addition, Bergmann created the 1.5 ml and 0.75 ml PEP centrifuge cups with their snap-on tight cover as convenient vessels for transferring fluid with the micropipette, which quickly impressed laboratories worldwide. A microcentrifuge complemented this new toolset, in which the micropipette was to become an integral part of enzymatic assays together with the Eppendorf photometer.

The revolutionary micropipette gained broad acceptance in Germany and throughout Europe but it took several years before it became standard equipment in the USA. Eppendorf failed to conquer this large market by focusing on technical perfection rather than on marketing. Eventually, Gilson Inc. (Middleton, WI, USA) realized

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BEKANNTMACHUNG DER ANMELDUNG UND AUSGABE DER AUSLEGESCHRIFT: 6. OKTOBER 1960
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STIMMT ÜBEREIN MIT AUSLEGESCHRIFT

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Es besteht seit langem das Bedürfnis, kleinste Flüssigkeitsmengen in der Größenordnung von 1 bis 100 Mikroliter schnell und ohne zu großen Aufwand an Sorgfalt und Konzentration zu pipettieren. Die erzielbare absolute Genauigkeit, vor allem aber die Reproduzierbarkeit bei Messungen mit dem gleichen Gerät, soll möglichst nicht geringer sein als bei der Abmessung größerer Flüssigkeiten, d. h. etwa 1% für die absolute Genauigkeit und etwa 0,5% bis 0,5‰ für die Reproduzierbarkeit. Für die heute häufig vorkommenden Versuche mit radioaktiven Substanzen oder für Fermentteste ist es außerdem sehr wichtig, daß das Säuberungsproblem so gelöst ist, daß man sehr schnell nacheinander die verschiedensten Lösungen pipettieren kann, ohne auch nur die allergeringste Spur an Substanz vom letzten Meßvorgang zu verschleppen.

Vorrichtung
zum schnellen und exakten Pipettieren
kleiner Flüssigkeitsmengen

Patentiert für:
Dr. med. Heinrich Schnitger,
Marburg/Lahn

Dr. med. Heinrich Schnitger, Marburg/Lahn, ist als Erfinder genannt worden

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Fig. 1 Fig. 2

Fig 3 | (Left) The patent application for the micropipette. The upper part of the first page and the introduction are shown. (Right) The design principle of the modern micropipette from the patent. In part 1, note the handle (3) pushing the piston (6). Its nose (3′) runs in a slit of the first concentric mobile cylinder. The piston (6) is spring-loaded and runs to the end of the housing (1). The inner cylinder is also loaded by a stronger spring (7), which can be pushed down with increased pressure when the nose (3′) hits the lower end of the slit. Part 2 shows the principle construction of the plastic tip, which receives an exact quantity of fluid when the handle held at the lower end of the slit is released.

its enormous market potential and created its own brand with a variable volume setting. Although the company copied the basic principles of Schnitger's invention and some further developments by Eppendorf, Gilson became successful in marketing the micropipette in the USA by exploiting loopholes and weaknesses in patent law. In particular, the variable volume pipette, already conceived by Schnitger in his patent application, quickly became popular among researchers.

> … Schnitger maintained that a single person is fundamentally more creative than a team…

The Marburg pipette was further accompanied by other, technically more intricate, inventions that Schnitger made to ease and accelerate the analysis of metabolites in tissue extracts. These included a novel fraction collector with up to eight parallel chromatography columns using Teflon rags to collect the samples. This allowed high-temperature oxidation of organic phosphates in special aluminium blocks; high-pressure multiple-piston pumps to drive fluids through 1-mm-wide and up to 8-m-long coiled ion exchange columns; and a special UV-micro-spectrophotometer with quartz optics to allow measurements of 10 μl samples (Schnitger et al, 1959). However, these and several other tools were not commercialized because of their sophisticated construction and a limited market.

It would not do justice to an inventor such as Schnitger if I did not mention his personal interests beyond the engineering of novel instruments. He was an unusual character and the focus of many, often humorous, stories. Although a friendly person, he showed no patience for mindless preoccupations and precisely defended his often extreme views. Acoustic perfection was one of his passions—he bought the best recording and amplifying equipment and designed a novel hi-fi loudspeaker. He was also very particular in preparing his food, and would take time-temperature profiles for cooking to optimal taste and weighed ingredients using an analytical balance purchased for this purpose. Another important activity concerned his special interest in measuring atmospheric conditions, which, he claimed, control personal mood. He measured atmospheric ionization by the highly variable ignition voltage of a mercury arc and, by recording the ignition voltage day and night every 10 min, he obtained records which he used to decide whether the time and day was good for productive activity.

More importantly though, Schnitger maintained that a single person is fundamentally more creative than a team, quoting his design of a coagulometer, where he had succeeded while other teams had failed. An independently thinking single brain, he claimed, could be more efficient in finding unconventional solutions and could be more creative than when wired to other brains in a team. Today, when research is done by increasingly larger teams, this 'antique' view may not only revive some nostalgic memories of the early days of molecular biology, but also demonstrates that there is still a place for individual inventors to make their revolutionary marks in science and technology.

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Martin Klingenberg is Professor Emeritus at the Institute of Physiological Chemistry at the Ludwig-Maximilians University, Munich, Germany.
E-mail: klingenberg@med.uni-muenchen.de

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